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Effect of Polyherbal formulation in Alloxan Induced diabetic rat

Sachin M. Mahajan*, Dheeraj T. Baviskar[2]. P.M. Chaudhari[1]
[1]Department of Pharmacognosy, K.V.P.S. Institute of Pharmaceutical education Boradi- 425 428 Dist-Dhule (M.S.) India.
[2]Department of Pharmacognosy KDGM Dr. Uttamrao Mahajan College of pharmacy Chalisgaon 424401 Dist-Jalgaon

ABSTRACT
The present study investigates the antihyperlipidemic and liver protective effect of single dose of Polyherbal formulations (PHF) containing alcoholic extracts of various parts like rhizomes of Curcuma caesia, Roxb, seeds of Caesalpenia bonduc, seeds of Citrullus lanatus , leaves of Gymnema sylvestre, stem of Tinospora cordiofolia, fruits of Withania coagulans and whole plant of Evolvulus alsinoide on alloxan-induced diabetic rat. On phytochemical investigation of PHF, reveals the presence of glycosides, triterpenoid, flavonoids and alkaloids, steroid, and tannins. It was observed that after 14th day of prolong treatment with Polyherbal formulation (400 mg/kg) and Glibenclamide (10mg/kg) lowers lipid level, inc insulin level and exerts liver protection by lowering the SGOT, SGPT. These findings suggested the antihyperlipidemic and Liver protective properties of the PHF(400 mg/kg) and thus help in preventing future complications of diabetes.
KEYWORDS: *Polyherbal Formulation, Alloxan induced, Antihyperlipidemic, Liver protective.*

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INTRODUCTION

Diabetes mellitus is a persistent metabolic disorder developed by an absolute or complete or relative lack of insulin along with decrease insulin activity that results in hyperglycemia and deviation in carbohydrate, fat and protein metabolism [1]. Diabetes is a major and growing public health problem throughout the world, with an estimated worldwide prevalence of 171 million people in 2000, expected to expand up to 366 million people by 2030[2]. In the three countries, China about 90 M around 9.3% of its population; India, about 61.3 million around 8.3% of its population; United States about 25.8 M around 8.3% of the population of the USA that has the most people living with type 2 diabetes[3]. It was observed that there is a prominent link between Type II diabetes and cardiovascular diseases which consist of a significant pattern of diabetic dyslipidemia, consists of low high-density lipoprotein (HDL), increased triglycerides [4], and abnormality in Glucose Homeostasis result is liver diseases or impairment in liver enzymatic function [5].

Around the era of 1300 A.D., "Sarangdhar Samhita" well-known ayurvedic literature highlighted the concept of polyherbalism. In the traditional system of Indian medicine, mostly mixed extracts of plants are elected over single plant for the development of polyherbal formulation to produce appropriate therapeutic effects [6]. Current synthetic anti-diabetic drug produces several side effects particularly, skin rash or itching, weight gain, gastrointestinal tract disturbances, low blood sugar level, etc.[7].

MATERIAL AND METHODS

Chemicals and reagents

All the chemicals used for the present study were analytical grade.

Collection of Plant Material:

The medicinal plants composing Polyherbal Formulation (PHF) were collected from leghapani near Toranmal in Satpuda hills, namely dried rhizome of *Curcuma caesia*, dried whole plant of *Evolvulus alsinoide*, seeds of *Citrullus lanatus,* leaves of *Gymnema sylvestra*, stems of *Tinospora cordiofolia* fruits of *Withania coagulans* Dunal (Solanaceae), seeds of *Caesalpenia bonduc* (Caesalpiniaceae) were

authenticated by Dr. D.A. Patil, Head of Department of Botany, S.S.V.P.S. art science and commerce College, Dhule.

Preparation of Extracts [8,9]

The collected plant material was dried under shade, such dried plants were pulverized by mechanical methods and passed through sieve no 80. Approximately 100 gm of each selected plant material were powdered and subjected to extraction with various solvents such as petroleum ether(40-60), ethanol by using soxhlet apparatus successively at room temperature. The extracts were filtered and concentrated at reduced pressure and lyophilized then stored carefully for further investigation.

Phytochemical investigation [8,10]

All the Preliminary qualitative phytochemical analysis of all the extracts was carried out by using standard conventional protocols.

Preparation of polyherbal formulation [11,12]

The evaporated lyophilized residue alcoholic extracts various selected parts of *Curcuma caesia, Evolvulus alsinoide*, *Citrullus lanatus, Gymnema sylvestra*, *Tinospora cordiofolia, Withania coagulance*, and *Caesalpenia bonduc* in the ratio of 1:1:1 were mixed in water and the different additives likeTween-80, Sodium CBC, methyl paraben, propyl paraben, Flavouring agent (Lemon oil) used for its better stability.

Animal.

Adult Wistar albino rats of either sex (180-250 g) were housed in standard temperature/humidity conditions and environment (12 h light/dark cycles) all sets of experiments. The rats were allowed to take standard pellet diet and water ad libitum all time except during the estimation of the behavioral parameters. The protocols for experimental were approved by the Institutional Animal Ethics Committee of R. C. PIPER Shirpur, Dist-Dhule, Maharashtra, India which was registered with Committee for control and supervision of experiments on animal (CPCSEA), Govt. of India.

Acute Toxicity Studies [13]

The acute oral toxicity study was carried out as per the guidelines set by Organization for Economic Co-operation and Development (OECD) guidelines 423. During the period of toxicity, animals were administered with single doses of in different groups and observed for mortality. In each and every step three animals were used in each group. The animals were under surveillance continuously for a period of 24 hours. A single oral administration of the dose from 300mg/kg body weight to 5000 mg/kg body weight in a different group of rats. There was no mortality observed at 5000mg/kg for the formulation. Therefore 5000mg/kg dose was considered as cut off dose so 1/10th and 1/5th of the maximum dose were selected.

Induction of diabetes [14,15]

Freshly prepared Alloxan monohydrate 150 mg/kg body weight in saline solution were administered prepared in by intraperitoneal route. After one hour of administration given a dose of alloxan monohydrate, animals were given feed ad libitum and 1 ml of 100mg/ml glucose I.P. to confirm hypoglycemia after 72 hours of alloxan injection. Induction of diabetes monitored on 3rd day by measuring glucose level. The blood glucose level of more than 200 to 350 mg/ml of blood was a selection of criteria for the experimental observation.

Evaluation of antihyperlipidemic activity on alloxan-induced diabetic rat of the Polyherbal formulation.

The Diabetic animals were randomly divided into six groups with 8 rats in each group and treated as follows for 14 days continuously orally. Normal group is administered with normal saline solution(saline 1ml p.o). Alloxan-induced diabetic rats were treated with water. Standard group receives Glibenclamide (10 mg/kg bw). Group 4 consists of diabetic rats receive Polyherbal Formulation (PHF 100 mg/kg bw p.o one's daily dose). Group 5 consists of diabetic rats who receive Polyherbal Formulation (PHF 200 mg/kg bw p.o one's daily dose). Group 6 consists of diabetic rats who receives Polyherbal Formulation (PHF 400 mg/kg bw p.o one's daily dose). Lipid level were monitored after of a daily single dose and at the end of 14 days for prolonged treatments.

Collection of blood sample

Blood samples of the fasted rats were collected on 14th day from Retro-orbital immediately with capillary tubes under ether anesthesia. Serum was separated by centrifugation method at 1500 rpm for 10 min.

Biochemical analysis

Animals were sacrificed by cervical decapitation under light ether anesthesia and blood was collected at the end of 14th day of experiment, serum was separated by centrifuging at 1500 rpm for 10 min. The serum was used for the assay of the biochemical parameters such as total cholesterol (TC), High-density lipoprotein-cholesterol (HDL-C), and triglycerides using the diagnostic kits (ERBA). The liver marker enzymes, such as SGOT and SGPT [16] were also estimated. Pancreas was excised from the animals, washed in ice-cold saline, and dried gently on the filter paper.

Estimation of total cholesterol, triglyceride and creatinine: The lipid profile parameters such as total cholesterol (Cholesterol oxidase- peroxidase method), HDL and serum triglyceride (GPO-POD Method) estimation were carried out fasting serum samples using commercial kits manufactured by ERBA

Estimation of SGPT, SGOT: The liver function test such as SGPT, SGOT were estimated by using kits manufactured by Crest Biosystems, India, Pvt. Ltd.

Histopathological study

The anatomized samples of pancreas from each group of diabetic animals were collected in 10% formalin-saline solution and stained with fluorescence dye (hemotoxylin and eosin) for preparation of section using a microtome and histopathological studies were carried out (Fig -1).

Statistical analysis

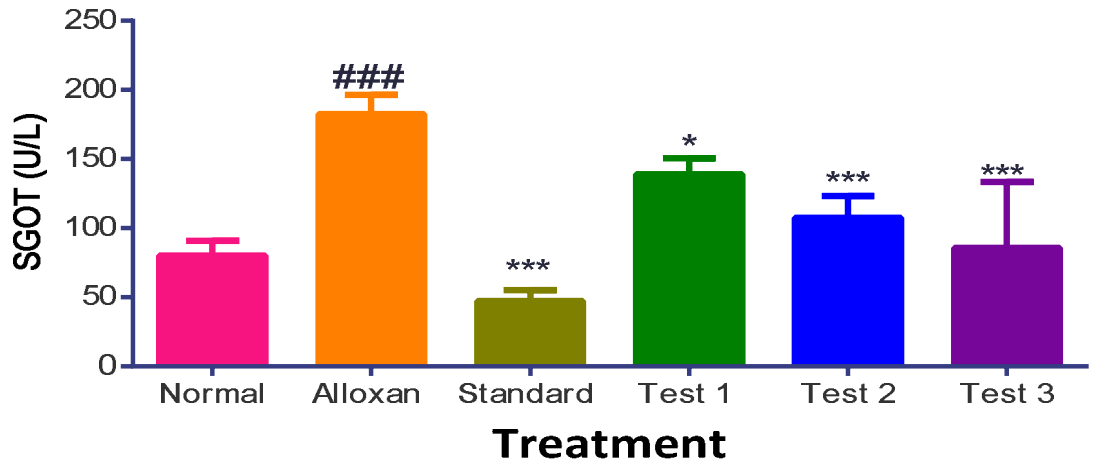
Values reported are mean ± standard error. The statistical analysis was carried out using analysis of variance, followed by Dunnet's ***t*** -test. ***P*** <0.001 were considered as significant.

RESULT

The phytochemical investigation showed that all the drug extracts contain mainly glycosides, triterpenoid, flavonoids and alkaloids, steroid and tannins. On the 14th day there was a statistically significant (P <0.001) increase in serum total cholesterol (189.1±13.96.), triglycerides (258.9±16.15), SGPT (179.7±12.88), SGOT (182.6±5.58) and decreased HDL (28.9±1.823) of Diabetic control group rats, as compared to normal control group rats [Table-1].

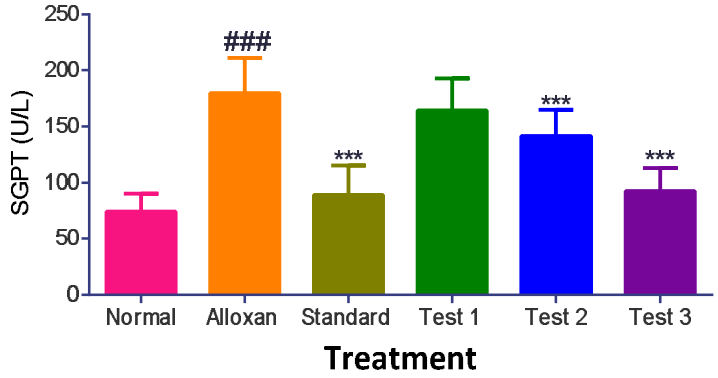
Although the repeated dose administration for 14 days of PHF (200 and 400 mg/kg/day) and Glibenclamide (10 mg/kg/day) in diabetic rats significantly (P < 0.001) reversed these change in plasma lipid profile when compared with diabetic control. Treatment with Glibenclamide, PHF 2 and PHF 3 for 14th days, significantly decreased total cholesterol (132.3±10.05), triglyceride(155.9±3.17), SGPT (92.60±8.37), SGOT (85.54±19.53) and significantly increased in HDL levels (52.31±1.192) when compared to Diabetic control group as far as the relative efficacy is concerned, PHF 2 and PHF 3 formulations showed more/comparable activity to Glibenclamide as well as diabetic control on all the biochemical parameters, but as far as overall efficacy is concerned for all parameters, PHF 400 mg /Kg is the best among all.

On the 14th day after insulin determination, it was found that there is stimulation of insulin in PHF 400 mg /Kg which is comparable to the standard [Table no.2].



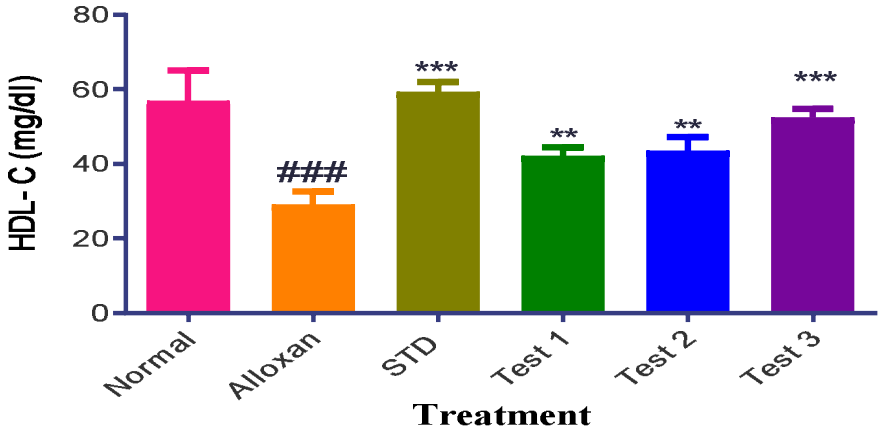
Data were analyzed by one-way analysis of variance (ANOVA) followed by Dunnett's post hoc test. ###p<0.001 as compared with normal rats, **p<0.01, ***p<0.001 as compared with Alloxan treated rats

Graph: 1 Effect of herbal formulation on serum SGOT level of Alloxan-induced diabetic rats during prolonged treatment



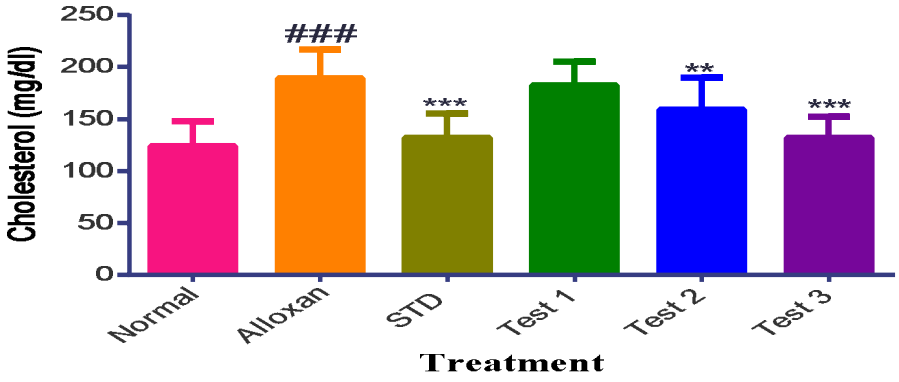
Data were analyzed by one-way analysis of variance (ANOVA) followed by Dunnett's post hoc test. ###p<0.001 as compared with normal rats, **p<0.01, ***p<0.001 as compared with Alloxan treated rats.

Graph 2: Effect of herbal formulation on serum SGPT level of Alloxan-induced diabetic rats during prolonged treatment



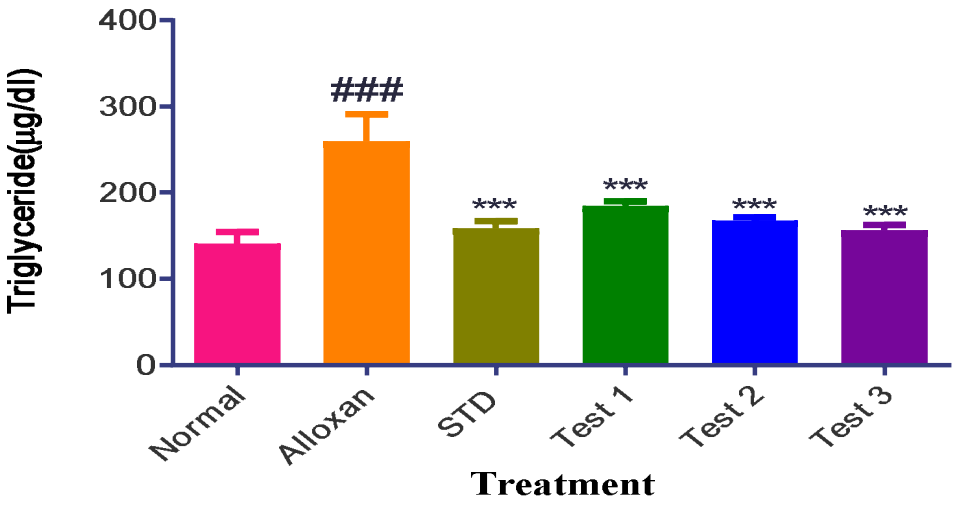
Data were analyzed by one-way analysis of variance (ANOVA) followed by Dunnett's post hoc test. ###p<0.001 as compared with normal rats, **p<0.01, ***p<0.001 as compared with Alloxan treated rats.

Graph 3: Effect of herbal formulation on serum HDL levelof Alloxan-induced diabetic ratsduring prolonged treatment



Data were analyzed by one-way analysis of variance (ANOVA) followed by Dunnett's post hoc test. ###p<0.001 as compared with normal rats, **p<0.01, ***p<0.001 as compared with Alloxan treated rats.

Graph 4: Effect of herbal formulation on serum cholesterol level of Alloxan induced diabetic rats during prolonged treatment



Data were analyzed by one-way analysis of variance (ANOVA) followed by Dunnett's post hoc test. ###p<0.001 as compared with normal rats, **p<0.01, ***p<0.001 as compared with Alloxan treated rats.

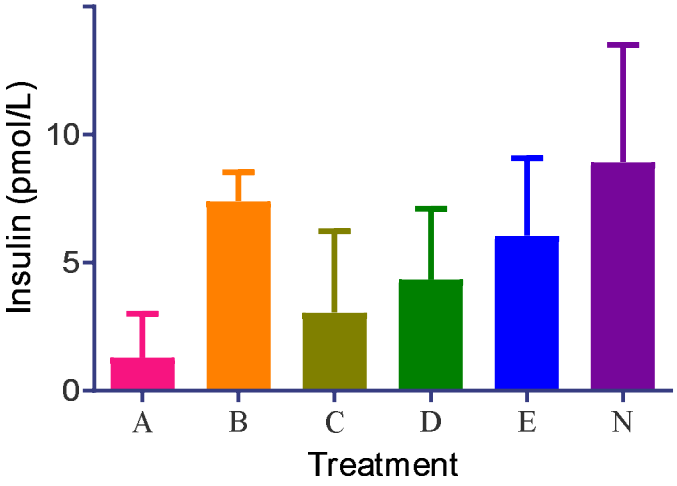
Graph: 5 Effect of herbal formulations on serum triglyceride level

Table: 1 Effect of herbal formulation on serum SGOT level, SGPT level, HDL level, triglyceride, cholesterol level on 14 th days

Groups	SGOT	SGPT	HDL level	Triglyceride	Cholesterol
Normal	79.81± 4.46	74.16± 6.57	56.73± 4.172	140.4± 7.06	123.6± 12.25
Alloxan	182.6± 5.58	179.7± 12.88	28.9± 1.823	258.9± 16.15	189.1± 13.96
STD	47.18± 3.13	89.04± 10.76	59.07± 1.463	157.9± 4.43	132.1± 11.71
Test 1(PHF 100 mg/kg)	139.2± 4.56	164.5± 11.58	42.06 ± 1.236	183.8± 3.227	182.6± 11.48
Test 2(PHF 200mg/kg	107.5± 6.305	141.6± 9.58	43.38± 1.94	167.3± 1.844	159.0± 15.49
Test 3(PHF 400mg/kg)	85.54± 19.53	92.60± 8.37	52.31± 1.192	155.9± 3.17	132.3± 10.05

Table: 2 Effect of herbal formulation on serum insulin on 14 th day

Unit	A(STD)	B (Alloxan)	C(Test I	D(Test II	E(Test III)	Normal
Pmol/L	1.2833	7.4	3.04286	4.32857	6.04286	8.92381



Graph 6: Effect of herbal formulation on serum insulin on 14 th day

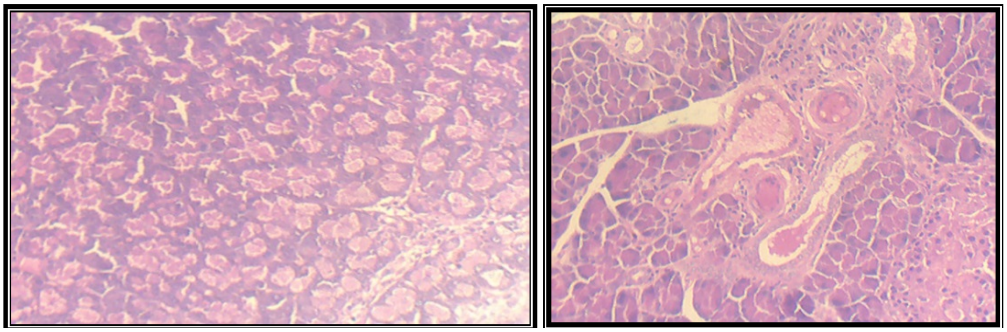


Fig1 a: Normal **Fig1 b: Alloxan control**

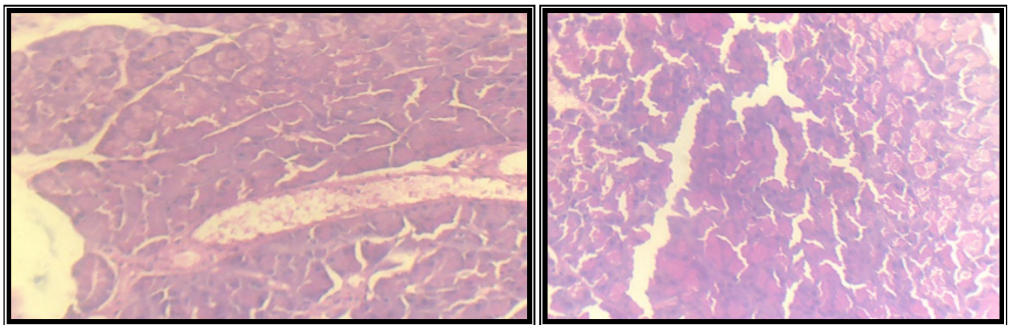


Fig1c: Test 1(PHF-100 mg/Kg) **Fig1 d: Test 2(PHF-200 mg/Kg)**

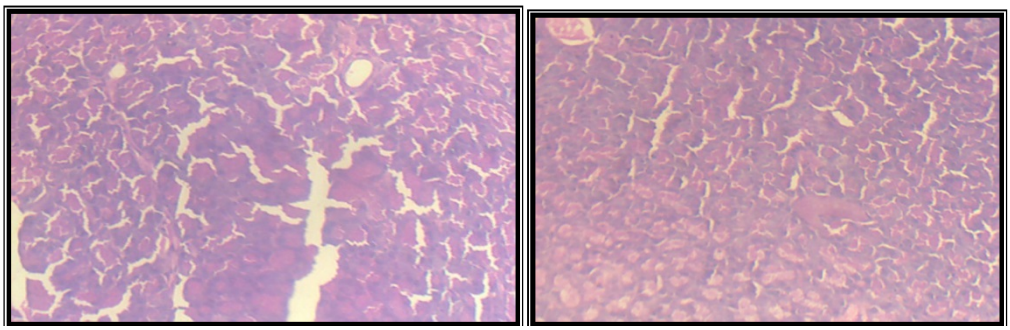


Fig 1e: Test 3 (PHF-400 mg/kg) **Fig1 f: Standard (Glibenclamide)**

Fig 1: Histopathology of Pancreas

DISCUSSION

The present manuscript discusses about the antihyperlipidemic activity of the polyherbal formulation on Alloxan-induced-diabetic rats. Phytochemical screening of Polyherbal formulation reveals (PHF-400mg/kg)) presence of flavonoids and tritepenoids, steroid and alkaloid. As per the concept in Ayurveda, synergy or antagonism by the homogenized herbal extracts in boosting hyperglycemia and dyslipidemia depended on the type and nature of individual herbal extract used in constituting herbal formulations. By implication, composition of various herbal extracts caused reconciliation in the absolute concentrations of the therapeutically active principles [17]. Alloxan monohydrate destroys the Beta cells of islets of langerhans of the pancreas and inhibit the production of insulin which affects to push glucose into the body tissues resulting high level of glucose decreased proteins content and increased cholesterol and triglycerides level in the blood[18]. The higher lipid levels seen in diabetic rats was due to increased mobilization of free fatty acids from peripheral depots and also due to lipolysis caused by hormones [19] . *Citrullus lanatus* decreasing intestinal absorption of lipids by promoting their fecal elimination [20]. *Withania coagulans* extracts exhibits antihyperglycemic effects through modulation of insulin and related enzyme activities in consonance with other studies demonstrated antihyperglycemic as well as protective effect in other organs apart from kidneys [21]. Antihyperlipidemic action of *Withania coagulans* extracts has also been reported in streptozotocin-induced diabetic rats. It shows hypolipidemic effects in triton-induced hypercholesterolemia due to its interference in synthesis, metabolism, and excretion of lipids. *Withania coagulans* extracts contain many withanolides and lactones which have been reported to show Cardiovascular benefits in dyslipidemia [22]. *Tinospora cordifolia* is very much beneficial in enhancing HDL cholesterol levels and lowering the LDL and VLDL cholesterol levels, thereby reveals its usefulness and therapeutic values. When we collectively look into the blood glucose levels and lipid profiles after the *Tinospora cordifolia* treatment in diabetic animals, it is evident that the anti- hyperglycemic activity of TC is re-sponsible for the controlling and correcting the altered lipids profile [23]. *Caesalpinia bonducella extracts*, has been reported to possess its antihyperlipidimic action by inhibiting the intestinal absorption of cholesterol and accelerating the catabolism of cholesterol [24]. Clinical and experimental evidence suggests that diabetes mellitus (DM) affects the liver in addition to blood vessels, kidneys, retina and nerves [25]. The liver is that the most important organ for the metabolism of medication and alternative toxicants. Any disease condition and or physical trauma results in the destruction of the liver cell facilitate impairment of the liver cell membrane permeability which is responsible for in the leakage of tissue contents into the blood resulting in the release of these intracellular enzymes into the blood. It has been observed that the liver is necrotized in diabetic rats which leads to increased activities of SGOT, SGPT enzymes as they leak from the liver cytosol into the blood stream [26]. In the present study, PHF-3 showed maximum effectiveness in decreasing the level of serum lipids is usually elevated in diabetes which represents a high-risk factor for coronary heart disease. It was found that, in a diabetic state, lipoprotein lipase is not activated in sufficient amount due to insulin deficiency resulting in hypertriglyceridemia. The treatment with Polyherbal (PHF-3) led to a significant decrease in total cholesterol, triglycerides, which implies that these formulations can reduce the complications of lipid metabolism and associated cardiovascular risk factors during diabetes. Polyherbal (PHF-3), the formulation has proved to be most effective in improving lipid metabolism in the present study as compared to Glibenclamide. Hepatotoxicity is another risk factor associated with oral hypoglycaemic on long term use. This risk factor can be minimized by reducing the dose of oral hypoglycaemic and using them in combination with herbal drugs. The Polyherbal (PHF-3) exhibited better results than Glibenclamide, for SGOT and SGPT levels. Though Polyherbal (PHF-3), formulation showed maximum efficacy for different biochemical parameters but looking to the results for all the parameters, it may be concluded that Polyherbal (PHF-3), was most effective in comparison to Polyherbal (PHF-2), Glibenclamide as well as diabetic control. There were no toxic effects during the14th days of study, regarding hepatotoxicity.

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CONFLICT OF INTEREST:

The authors declare no conflict of interest.

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